

WEST Search History

DATE: Wednesday, January 29, 2003

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result set

DB USPT,PGPB,JPAB,DWPI; PLUR YES; OP ADJ

L9	L5 and PTN	0	L9
L8	L5 and AOX1	4	L8
L7	L5 and Pichia	22	L7
L6	L5 and MK	1	L6
L5	11 or 12 or 13 or L4	81	L5
L4	MFalpha1 or alpha pheromone or alpha-pheromone	35	L4
L3	alpha 1 factor	5	L3
L2	MF alpha 1	44	L2
L1	MF alpha 1 same saccharomyces cerevisiae	12	L1

L8	L5 and AOX1	4	L8
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L7	L5 and Pichia	22	L7
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L6	L5 and MK	1	L6
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L5	l1 or l2 or l3 or L4	81	L5
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L4	MFalpha I or alpha pheromone or alpha-pheromone	35	L4
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L3	alpha 1 factor	5	L3
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L2	MF alpha 1	44	L2
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L1	MF alpha 1 same saccharomyces cerevisiae	12	L1
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END OF SEARCH HISTORY

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NEWS 9 Jun 03 New e-mail delivery for search results now available
NEWS 10 Jun 10 MEDLINE Reload
NEWS 11 Jun 10 PCTFULL has been reloaded
NEWS 12 Jul 02 FOREGE no longer contains STANDARDS file segment
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NEWS 19 Aug 19 Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20 Aug 19 IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21 Aug 19 The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22 Aug 26 Sequence searching in REGISTRY enhanced
NEWS 23 Sep 03 JAPIO has been reloaded and enhanced
NEWS 24 Sep 16 Experimental properties added to the REGISTRY file
NEWS 25 Sep 16 CA Section Thesaurus available in CAPLUS and CA
NEWS 26 Oct 01 CASREACT Enriched with Reactions from 1907 to 1985
NEWS 27 Oct 21 EVENTLINE has been reloaded
NEWS 28 Oct 24 BEILSTEIN adds new search fields
NEWS 29 Oct 24 Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 30 Oct 25 MEDLINE SDI run of October 8, 2002
NEWS 31 Nov 18 DKILIT has been renamed APOLLIT
NEWS 32 Nov 25 More calculated properties added to REGISTRY
NEWS 33 Dec 02 TIBKAT will be removed from STN
NEWS 34 Dec 04 CSA files on STN
NEWS 35 Dec 17 PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 36 Dec 17 TOXCENTER enhanced with additional content
NEWS 37 Dec 17 Adis Clinical Trials Insight now available on STN
NEWS 38 Dec 30 ISMEC no longer available
NEWS 39 Jan 13 Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 40 Jan 21 NUTRACEUT offering one free connect hour in February 2003
NEWS 41 Jan 24 PHARMAML offering one free connect hour in February 2003

NEWS EXPRESS January 6 CURRENT WINDOWS VERSION IS V6.01a,
CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0b(JP),
AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
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=> s alpha pheromone or MFalpha1 or MF alpha 1 or MF alpha1 or alpha 1 factor
L1 494 ALPHA PHEROMONE OR MFALPHA1 OR MF ALPHA 1 OR MF
ALPHA1 OR ALPHA
1 FACTOR

=> s l1 and MK
L1 1 L1 AND MK

=> d sib abs

L2 ANSWER 1 OF 1 CAPLUS COPYR GHT 2003 ACS
AN 2000 133856 CAPLUS
DN 132 179658
TI Mass secretion/expression system for unglycosylated human ***Mk***
family proteins in methylotrophic yeast
IN Murasugi, A.; Asami, Yukio; Kido, Isao; Kumai, Hideshi
PA Meiji Milk Products Co., Ltd., Japan
SI PCT Int Appl. 58 pp
CCDEN: P1KX02
DI Patent
LA Japanese
FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
PI WO 23000087	A1	20000224	WO 1999-JP4332	19990810
W AU, CA, CN, JP, KR, US				
RW AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2339350	AA	20000224	CA 1999-2339350	19990810
AU 9950674	A1	20000306	AU 1999-50674	19990810
EP 1106697	A1	20010613	EP 1999-935123	19990810
R AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, T, LV, FI, RO				
PRAI JP 1999-236621	A	19990810		
JP 1999-84583	A	19990326		
WO 1999-JP4332	W	19990810		
AB Large-scale secretion/expression system for unglycosylated human ***Mk*** family proteins in methylotrophic yeast, Pichia pastoris is described. The expression vector consists of a mature human ***Mk*** family protein coding region cDNA attached to Saccharomyces cerevisiae ***alpha*** ***1*** ***factor*** signal sequence under the control of a Pichia pastoris methanol-inducible alc oxidase gene (AOX1) promoter, a transcription termination sequence, and an origin of replication. Unglycosylated human ***Mk*** protein and PTN protein were mass produced in Pichia pastoris GS115 and SMD118 strains, and the expressed proteins demonstrated their bioactivity for facilitating growth of mouse embryo fibroblast NIH3T3.				

RE CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> c js
'JS' IS NOT A VALID FORMAT FOR FILE 'CAPLUS'

The following are valid formats

ABS G1 and AB
ALL B B AB, IND, RE
APPS AI, PRAI
BIB AN, p js Bibliographic Data and PI table (default)
CAN List of CA abstract numbers without answer numbers
CBIB AN, plus Compressed Bibliographic Data
DALL ALL, delimited (end of each field identified)
DMAX MAX, delimited for post-processing
FAM AN, PI and PRAI in table, plus Patent Family data
FBIB AN, BIB, plus Patent FAM
INE Indexing data
IPC International Patent Classifications
MAX ALL, plus Patent FAM, RE
PATS PI, SO
SAM CC, SX, TI, ST, IT
SCAN CC, SX, TI, ST, IT (random display, no answer numbers, SCAN must be entered on the same line as the DISPLAY, e.g., D SCAN or DISPLAY SCAN)
STD BIB, IPC, and NCL
IABS ABS, indented with text labels
JAL ALL, indented with text labels
IBIB BIB, indented with text labels
IMAX MAX, indented with text labels
ISTD STD, indented with text labels
OBIB AN, plus Bibliographic Data (original)

OIBIB ----- OIBIB, indented with text labels

SBIB ----- BIB, no citations

SIBIB ----- IBIB, no citations

HIT ----- Fields containing hit terms

HITIND ----- IC, ICA, ICI, NCL, CC and index field (ST and IT) containing hit terms

HITRN ----- HIT RN, its text modification

HITSTR ----- HIT RN, its text modification, its CA index name, and its structure diagram

HITSEQ ----- HIT RN, its text modification, its CA index name, its structure diagram, plus NTE and SEQ fields

FHITSTR ----- First HIT RN, its text modification, its CA index name, and its structure diagram

FHITSEQ ----- First HIT RN, its text modification, its CA index name, its structure diagram, plus NTE and SEQ fields

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CAN ----- List of CA abstract numbers without answer numbers

CBIB ----- AN, plus Compressed Bibliographic Data

DALL ----- ALL, delimited (end of each field identified)

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FBIB ----- AN, BIB, plus Patent FAM

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MAX ----- ALL, plus Patent FAM, RE

PATS ----- PI, SO

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STD ----- BIB, IPC, and NCL

IABS ----- ABS, indented with text labels

IALL ----- ALL, indented with text labels

IBIB ----- BIB, indented with text labels

IMAX ----- MAX, indented with text labels

ISTD ----- STD, indented with text labels

OIBIB ----- AN, plus Bibliographic Data (original)

OIBIB ----- OIBIB, indented with text labels

SBIB ----- BIB, no citations

SIBIB ----- IBIB, no citations

HIT ----- Fields containing hit terms

HITIND ----- IC, ICA, ICI, NCL, CC and index field (ST and IT) containing hit terms

HITRN ----- HIT RN, its text modification

HITSTR ----- HIT RN, its text modification, its CA index name, and its structure diagram

HITSEQ ----- HIT RN, its text modification, its CA index name, its structure diagram, plus NTE and SEQ fields

FHITSTR ----- First HIT RN, its text modification, its CA index name, and its structure diagram

FHITSEQ ----- First HIT RN, its text modification, its CA index name, its structure diagram, plus NTE and SEQ fields

KWIC ----- Hit term plus 20 words on either side

OCC ----- Number of occurrence of hit term and field in which it occurs

To display a particular field or fields, enter the display field codes. For a list of the display field codes, enter HELP DFIELDS at an arrow prompt (=>). Examples of formats include TI, TI, AU, BIB, ST, TI, IND, TI, SO. You may specify the format fields in any order and the information will be displayed in the same order as the format specification

All of the formats (except for SAM, SCAN, HIT, HITIND, HITRN, HITSTR, FHITSTR, HITSEQ, FHITSEQ, KWIC, and OCC) may be used with DISPLAY ACC to view a specified Accession Number

ENTER DISPLAY FORMAT (BIB) b/b

L2 ANSWER 1 OF 1 CAPLUS, COPYRIGHT 2003 ACS

AN 2000 133856 CAPLUS

DN 132 179658

TI Mass secretion/expression system for unglycosylated human ***MK*** family proteins in methylotrophic yeast

IN Murasugi, Akira; Asami, Yukio; Kudo, Isao; Kumai, Hideshi

PA Meiji Milk Products Co., Ltd., Japan

SO PCT Int. Appl. 58 pp

CODEN PIKXD2

DT Patent

LA Japanese

FAN CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 2000/09718	A1	20000224	WO 1999 JP4332	19990810
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W AU, CA, CH, JP, KR, US

RW AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

CA 2339350 AA 20000224 CA 1999-2339350 19990810

AU 9950674 A1 20000306 AU 1999-50674 19990810

EP 1106697 A1 20010613 EP 1999-935123 19990810

R AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, L LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRAI JP 1998-236621 A 19980810

JP 1999-84583 A 19990326

WO 1999 JP4332 W 19990810

RE CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOWL' ENTERED AT 13 06 28 ON 29 JAN 2003)

FILE 'BIO S, EMBASE, CAPLUS' ENTERED AT 13 06 37 ON 29 JAN 2003

L* 494 S A, PHA PHEROMONE OR MFALPHA1 OR MF ALPHA 1 OR MF

ALPHA1 OR ALP

L2 1 S L1 AND MK

=> s l1 and (methylotrophic yeast or Pichia)

L3 19 L1 AND (METHYLOTROPHIC YEAST OR PICHIA)

=> d up rem l3

PROCESSING COMPLETED FOR L3

L4 15 DUP REM L3 (4 DUPLICATES REMOVED)

=> d bib abs 1-

YOU HAVE REQUESTED DATA FROM 15 ANSWERS - CONTINUE? Y(N) y

L4 ANSWER 1 OF 15 CAPLUS, COPYRIGHT 2003 ACS

AN 2002 575222 CAPLUS

DN 137 136056

TI Combinatorial expression libraries with individual members of the library containing concatamers of expression cassettes

IN Goosmith, Neil; Sorensen, Alexandra M P; Santana, Nielsen, Soren V S

PA Evolve Biotech A/S, Den

SO PCT Int. Appl. 115 pp

CODEN PIKXD2

DT Patent

LA English

FAN CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 2002/59297	A2	20020801	WO 2002-Dr.56	20020125
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W AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,

LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, NZ, OM, PH,

PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,

UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,

TJ, TM

RW, GH, GM, KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZM, ZW, AT, BE, CH,

CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,

BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI DK 2001-128 A 20010125

DK 2001-673 A 20010501

US 2001-300863 P 20010627

AB Combinatorial gene expression libraries in which recombination between individual sequences can take place within an individual cell and methods of constructing such libraries are described. Each member of the library contains a large no. of expression cassettes that are randomly selected from a pool of cassettes during the construction of the library. Individual expression cassettes are flanked by a common pair of restriction sites and have the same promoter and terminator to regulate expression of the cloned inserts. The library of concatamers is created from a library of individual clones. This primary library, typically a cDNA library, has the individual cassette and its flanking restriction sites flanked by a second pair of restriction sites. The cassettes are released from the library and ligated into concatamers that are then cloned into a vector capable of stabilizing large inserts, esp. artificial

chromosomes. The variability within the combinatorial library can be increased by using cDNA libraries from multiple sources. Cassettes within the library are free to recombine with one another to create genes encoding novel activities or functions that can be identified by selection or screening. Such libraries are useful in discovery of novel or modified metabolic pathways leading to the prodn. of novel compds. for e.g. drug discovery and to the prodn. of known compds. in novel quantities or in novel compartments of the cells. The expression libraries in particular are composed of host cells capable of coordinated and controllable expression of large nos. of heterologous genes in the host cells.

L4 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2003 ACS
AN 2002 575221 CAPLUS

DN 137 136055

TI Combinatorial expression libraries with individual members of the library containing concatemers of expression cassettes

IN Goldsmith Neil, Sorensen, Alexandra M P, Santana Nielsen, Soren V S, Naestby, Michael

PA Evolve Biotech A/S, Den

SO PCT Int. Appl., 124 pp

CODEN PIKAD2

DT Patent

LA English

FAN CNT 1

PATENT NO	KIND DATE	APPLICATION NO	DATE
PI WO 2002059296	A2	20020801	WO 2002-DK55
			20020125

W AE, AG, AL, AM, AT, A J, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAIDK 2001 127 A 20010125

US 2001-301022P P 20010627

AB Combinatorial gene expression libraries in which individual clones contain large nos. of expression cassettes and methods of constructing such libraries are described. Each member of the library contains a large no. of expression cassettes that are randomly selected from a pool of cassettes during the construction of the library. Individual expression cassettes are flanked by a common pair of restriction sites and have the same promoter and terminator for uniform regulation of expression of the cloned inserts. The library of concatemers is created from a library of individual clones. This primary library, typically a cDNA library, has the individual cassette and its flanking restriction sites flanked by a second pair of restriction sites. The cassettes are released from the library and ligated into concatemers that are then cloned into a vector capable of stabilizing large inserts, esp. artificial chromosomes. The variability within the combinatorial library can be increased by using cDNA libraries from multiple sources. Such libraries are useful in discovery of novel or modified metabolic pathways leading to the prodn. of novel compds. for e.g. drug discovery and to the prodn. of known compds. in novel quantities or in novel compartments of the cells. The expression libraries in particular are composed of host cells capable of coordinated and controllable expression of large nos. of heterologous genes in the host cells.

L4 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 2002 10523 CAPLUS

DN 136 55975

TI Termit defense termicin and cDNA and their use in protection of plants from phytopathogenic fungi

IN Lamberty, Yvonne Bulet, Philippe, Latorse, Marie pascale, Hoffmann, Jules

PA Rhodia, F

SO PCT Int. Appl., 34 pp

CODEN PIKAD2

DT Patent

LA French

FAN CNT 1

PATENT NO	KIND DATE	APPLICATION NO	DATE
PI WO 200200706	A2	20020103	WO 2001-FR2028
			20010627

W AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

FR 2810993 A1 20020104 FR 2000-8374 20000629

FR 2810993 B1 20020823

PRAID FR 2000-8374 A 20000629

AB The invention concerns an antimicrobial peptide of the family of defensins, in particular antifungal, called termicin, DNA encoding said peptide, vectors contg. them for transforming a host organism and the

method for transforming said organism. The invention also concerns transformed organisms, in particular yeast, or plant cells and plants, the termicin produced by the transformed plants providing them with resistance to fungus-mediated diseases. Thus, the cDNA for Pseudocanthotermes spingeri termicin was expressed in *Saccharomyces cerevisiae*. The yeast ***MF*** **alpha*** **1*** promoter, prepro- sequence, and terminator were used to control expression and protein secretion. The recombinant termicin exhibited antifungal activity against *Cercospora fimensis*, *Botrytis cinerea*, *Septoria nodorum*, *S. tritici*, *Rhizoctonia solani*, *Fusarium graminearum*, and *F. nivale*.

L4 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2003 ACS
AN 2002 533549 CAPLUS

TI Glutamic acid and alanine spacer is not necessary for removal of ***MF*** **alpha*** **1*** signal sequence fused to the human growth hormone produced from ***Pichia*** pastoris

AU Eurwlaichtr, Lily, Roytrakul, Sittruk, Suprasongsin, Chittiwat, Mantchotpisit, Pennapa, Panyim, Sakol

CS Institute of Molecular Biology and Genetics, BIOTEC Training Center for Genetic Engineering and Biotechnology, Mahidol University, Nakhonpathom, 73170, Thailand

SO World Journal of Microbiology & Biotechnology (2002), 18(6), 493-498

CODEN WJMBEY, ISSN 0959-3993

PB Kluwer Academic Publishers

DT Journal

LA English

AB Human growth hormone (hGH) cDNA was synthesized using codons preferred by

Escherichia coli, except for the first 20 amino acids, which were changed to that preferred by *Saccharomyces cerevisiae* and ***Pichia*** pastoris. Polymerase chain reaction (PCR) overlapping approach was employed to create synthetic hGH without glutamic acid-alanine (glu-ala), or with one and two glu-ala spacers (hGH1 and hGH2, resp.). The necessity of a glu-ala spacer in the cleavage of *S. cerevisiae* alpha mating factor-1 (***MF*** **alpha*** **1***) secretion signal from the synthetic hGH was also investigated. Three types of hGH constructs were integrated into *P. pastoris* genome, the zeocin-resistant transformants were selected and expression of hGH was detd. A 22-kDa band of secreted hGH was further detd. by N-terminal peptide sequencing. The result suggested that the removal of glu-ala from the hGH1 and hGH2 was not efficient and only the hGH construct showed the complete cleavage of the signal sequence, giving a similar N-terminus as the mature hGH. hGH expression was optimized to increase the yield of the protein from the hGH construct (no glu-ala) to 190 mg/l from a 10-mL induction medium.

RE CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 2002 633226 CAPLUS

DN 137 336761

TI Strain and process development for the production of human cytokines in *Hansenula polymorpha*

AU Degelmann, Adelheid, Muller, Frank, Sieber, Heike, Jenzelewski, Volker;

Suckow, Manfred, Strasser, Alexander W. M., Gellissen, Gerd

CS Rhein Biotech GmbH, Dusseldorf, 40595, Germany

SO FEMS Yeast Research (2002), 2(3), 349-361

CODEN FYREAG, ISSN: 1567-1356

PB Elsevier Science B.V.

DT Journal

LA English

AB The early status of strain development for the prodn. of interleukin (IL)-6, IL-8, IL-10, and interferon (IFN) gamma is described. The general approach to generating such strains was to amplify gene sequences encoding the mature forms of the various cytokines by PCR from com. available cDNA sources. The design of the amplificates allowed an in-frame fusion to an ***MF*** **alpha*** **1*** leader segment contained in two basic expression vectors, pFPM121- ***MF*** **alpha*** **1*** and pTPSMT- ***MF*** **alpha*** **1***. The two vectors differ in that one harbors the methanol-inducible FMD promoter and the other the constitutive TPS1 promoter as control elements for heterologous gene expression. The most advanced process development example is that of IFN alpha-2a. Here, the MOX promoter derived from another key gene of methanol metab. is used for expression control. The successful development of a prodn. process for *Hansenula polymorpha*-derived IFN alpha-2a is summarized. This was achieved by combining genetic engineering of suitable prodn. strains with improved processing capabilities for the secreted cytokine, and by purifn. procedures from cultures grown in yeast ext.-peptone-glycerol-based media.

RE CNT 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INDUPLICATE

AN 2002 634408 BIOSIS

DN PREV200200634408

TI Production of IFNalpha-2a in *Hansenula polymorpha*

AU Mueller, Frank, Tiede, Anni, Wasch, Dorothea, Muehle, Christine, Mueller,

Frank, Seigelhofer, Mauricio, Pesce, Analia, Jenzelewski, Volker,

Gellissen, Gerd (1)

CS (1) Rhein Biotech GmbH, Eichsfelder Str. 11, 40595, Dusseldorf

g.gelissen@rheinbiotech.de Germany
SO Process Biochemistry (September, 2002) Vol. 38, No. 1, pp. 15-25
http://www.elsevier.com/locate/procbio print
ISSN 1359-5113

DT Article

LA English

AB A DNA sequence coding for IFN α 2a was expressed in the ***methylotrophic*** ***yeast*** *Hansenula polymorpha* from a strong inducible promoter element derived from the MOX gene, a key gene of the methanol metabolism pathway. For secretion the coding sequence was fused to the KEX2 recognition site of the *S. cerevisiae*-derived ***MF α 1*** prepro-leader. To a large extent the secreted molecules were found to be incorrectly processed from the precursor molecule exhibiting N-terminal extensions of the mature protein. Correct processing was achieved when co-expressing a *S. cerevisiae*-derived KEX2 gene from its native promoter. Undesirable proteolytic cleavage at additional dibasic sites of the protein sequence could be minimised when optimising fermentation conditions. A pH/pO₂-controlled C-source feeding mode was applied to fermentations on a 1.5-101 scale. In cultures of a transformant strain harbouring 30 copies of the IFN expression cassette a productivity of 350 mg/l could be obtained. Various capture procedures were found to be impaired when using a standard synthetic medium for culturing. Binding to ion exchange and hydrophobic interaction matrices was regained when using a modified YPG-based culture medium.

L4 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 2000 133856 CAPLUS

DN 132 179658

T Mass secretion/expression system for unglycosylated human MK family proteins in methylotrophic yeast

IN Murasugi, Akira, Asami, Yukio, Kido, Isao, Kumai, Hideshi

PA Meiji Milk Products Co., Ltd., Japan

SO PCT Int. Appl., 58 pp

CODEN PDXD2

DT Patent

LA Japanese

FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
PI WO 2000/09718	A1	20000224	WO 1999-JP4332	19990810
W AL, CA, CN, JP, KR, US				
RW AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2339350	AA	20000224	CA 1999-2339350	19990810
AU 9950674	A1	20000306	AU 1999-50674	19990810
EP 1066847	A1	20010613	EP 1999-935123	19990810
R AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE, PT, IE, SI, LT, LV, FI, RO				
PRAI JP 1998-236621	A	19980810		
JP 1999-84583	A	19990326		
WO 1999-JP4332	W	19990810		

AB Large-scale secretion/expression system for unglycosylated human MK family proteins in methylotrophic yeast ***Pichia*** pastons is described. The expression vector consists of a mature human MK family protein coding region (CD_{1A}) attached to *Saccharomyces cerevisiae* ***alpha*** ***1*** ***factor*** signal sequence under the control of a ***Pichia*** *pastoris* methanol-inducible alc oxidase gene (AOX1) promoter, a transcription termination sequence, and an origin of replication. Unglycosylated human MK protein and PTN protein were mass produced in ***Pichia*** *pastoris* GS115 and SMD118 strains, and the expressed proteins demonstrated their biological activity for facilitating growth of mouse embryo fibroblast NIH3T3.

RE CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 2000 457379 CAPLUS

DN 133 84755

T Recombinant growth factors with the biological activity of G-CSF (Granulocyte Colony Stimulating Factor)

IN Fischer, Johannes, Wernet, Peter, Gellissen, Cerd, Weydemann, Ulrike,

Jenze, Pawel, Volker, Piontek, Michael, Strasser, Alexander W

PA Rhein-Biotech Gesellschaft fuer Neue Biotechnologische Prozesse und Produkte, Germany

SO Ger Offen., 22 pp

CODEN GWXXB

DT Patent

LA German

FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
PI DE 19860801	A1	20000706	DE 1998-19860801	19981230
WO 2000/040727	A2	20000703	WO 1999 EP10466	19991229
WO 2000/040727	A3	20001026		
W AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, GR, HU, ID, IL, IN, IS, JP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
PRAI DE 1998-19860801	A	19981230		

AB The invention provides growth factors with granulocyte colony-stimulating activity as well as nucleic acid molecules which comprise sequences coding for such growth factors. Moreover the invention concerns a procedure for manufacturing the growth factors of the invention, pharmaceutical compositions comprising proteins or nucleic acids according to the invention and their therapeutic use. The growth factors possess cytokine activities of differing expression and are able to stimulate maturation of blood cells. It is intended in particular to use the molecules of the invention for treating diseases and injuries which are associated with a lack of blood cells, e.g. cancer, leukemia, severe burns, opportunistic infections, and bone marrow transplants.

RE CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 1999 673020 CAPLUS

DN 131 308932

T Helicidin, chimeric helicidin-encoding genes, and transgenic plants resistant to fungi

IN Lamberty, Mireille, Bulet, Philippe, Brookhart, Gary, Lee, Hofmann, Jules

PA Rhone-Poulenc Agro, Fr

SO PCT Int. Appl., 68 pp

CODEN PDXD2

DT Patent

LA French

FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
PI WO 9652053	A1	19991021	WO 1999-FR843	19990412
W AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GR, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NC, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, BG, KZ, MD, RU, TJ, TM				
RW GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
FR 2777568	A1	19991022	FR 1998-4933	19980415
FR 2777568	B1	20021031		
CA 2325658	AA	19991021	CA 1999-2325658	19990412
AU 9931525	A1	19991101	AU 1999-31525	19990412
BR 9903745	A	20001226	BR 1999-9745	19990412
EP 071757	A1	20010131	EP 1999-913384	19990412
R AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001511260	T2	20020416	JP 2000-543601	19990412
NO 2000005173	A	20001215	NO 2000 5173	20001013
PRAI FR 1998-4933	A	19980415		
WO 1999-FR843	W	19990412		

AB The invention concerns helicidin, a DNA sequence coding for helicidin, a vector conferring it for transforming a host organism and the transformation method. The invention concerns helicidin as medicine, in particular for treating fungal infections. More particularly it concerns the transformation of plant cells and plants, the helicidin produced by the transformed plants ensuring their resistance to diseases, in particular diseases of fungal origin. Thus, helicidin was prepared with recombinant *Saccharomyces cerevisiae* and its activity against fungi and yeast demonstrated. Transgenic tobacco producing helicidin were prepared. The helicidin was not affected by the plant proteases and retained its antifungal activity for *Borytis cinerea*. Mice injected i.v. with 10 mg helicidin/kg displayed no evidence of toxicity.

RE CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 1998 479625 CAPLUS

DN 129 138045

T Recombinant preparation of N-terminally extended heterologous proteins in yeast with improved yield

IN Kjeldsen, Thomas, Borglum, Havelund, Svend, Petersson, Annette, Frost,

Balschmidt, Per

PA Novo Nordisk A/S, Den

SO PCT Int. Appl., 30 pp

CODEN PDXD2

DT Patent

LA English

FAN CNT 4

PATENT NO	KIND	DATE	APPLICATION NO	DATE
PI WO 9828429	A1	19980702	WO 1997-D-581	19971218
W AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GR, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, BG, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9878737	A1	19980717	AU 1998-78737	19971218
EP 946735	A1	19991006	EP 1997-948751	19971218

R AT BE CH DE DK ES FR GB GR IT LI LU NL SE PT IE
SI LT LV FI RO
JP 2001507574 T2 20010612 JP 1998-528258 19971218
PRAI DK 1996-1482 A 19961220
WO 1997-0K581 W 19971218
AB Disclosed is a method for the recombinant prep in yeast of heterologous proteins having inserted N-terminal extension EEEGPK to improve its fermn yield and protect against dipeptidyl aminopeptidase processing. The protein is prepd by expression from a plasmid of a DNA sequence encoding SP-LP-EEGPK-protein (SP=signal peptide LP=leader peptide) in transgenic yeast. Demonstrated was the expression of N-terminally extended insulin precursor EEEGPK-MI3 in *Saccharomyces cerevisiae* strain MT663 by using yeast YAP3 (yeast aspartic protease 3) signal peptide and synthetic LA19 leader peptide

RE CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL C TATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 1998 65528 CAPLUS

DN 128 138818

TI Manufacture of proinsulin and insulin and their analogs using a proteinase-deficient yeast expression host

IN Egel-Mitani, Michi, Brandt Jakob, Vad Knud

PA Novo Nordisk A/S, Den., Egel-Mitani, Michi, Brandt, Jakob, Vad, Knud

SO PCT Int Appl, 32 pp

CODEN PI*02

DT Patent

LA English

FAN CNT 1

PATENT NO	KIND DATE	APPLICATION NO	DATE
PI WO 9801473	A1 19980115	WO 1997-0K297	19970704

W AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RC RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW AM AZ BY KG KZ MD RJ TJ TM RW GH, H, C, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR GB, GR, IE, IT, LI, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9732553 A1 19980202 AU 1997-32553 19970704

PRAI DK 1996-748 19960705

WO 1997-0K297 19970704

AB Yeast expression hosts lacking the YAP3 proteinase are used to manu insulin, proinsulin, their analogs or related proteins (e.g. IGF-1). The preferred host is *Saccharomyces cerevisiae*, but other yeasts, e.g. *Pichia*, *Kluyveromyces*, may also be suitable. The host may also be deficient in other proteinases, e.g. BAR1, STE13. *S. cerevisiae* with the YAP3 gene insertionally inactivated with a URA3 deletion allele was constructed by std. methods. Manuf. of a proinsulin analog in such a host increased yields by 167-371% over control cells depending upon the precursor construct used

L4 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC DUPLICATE

2

AN 1996 75259 BIOSIS

DN PREV1996-6647394

TI High-level secretion of hirudin by *Hansenula polymorpha*-authentic processing of three different preprohirudins

AU Weydemann L (1), Keup P, Piontek M, Strasser A, W M, Schweden, J, Gellissen G, Janowicz Z A

CS (1) Rhein Biotech GmbH, Eichsfelder Str. 11, D-40595 Duesseldorf Germany

SO Applied Microbiology and Biotechnology, (1995) Vol 44, No 3-4, pp 377-385

ISSN 0175-7598

DT Article

LA English

AB A DNA sequence coding for a subtype of the hirudin variant HVI was expressed in the *methylophilic* *yeast* *Hansenula polymorpha* from a strongly inducible promoter element derived from a gene of the methanol metabolism pathway. For secretion, the coding sequence was fused to the KEX2 recognition site of three different prepro segments engineered from the *MF* *alpha* *gene* of *Saccharomyces cerevisiae*, the glucosylase (GAM) *gene* of *Schwannomyces*

occidentalis and the gene for a crustacean hyperglycemic hormone from the shore crab *Carcinus maenas*. In all three cases, correct processing of the precursor molecule and efficient secretion of the mature protein were observed. In fermentations on a 10-l scale of a transformant strain harbouring a *MF* *alpha* *hirudin-gene* fusion yields in the range of grams per litre could be obtained. The majority of the secreted product was identified as the full-length 65-amino-acid *hirudin*. Only small amounts of a truncated 63-amino-acid product, frequently observed in *S. cerevisiae*-based expression systems, could be detected

L4 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 1995 87198 CAPLUS

DN 122 2781

TI Use of the proform of defensin A in the secretion of heterologous proteins from yeast cells

IN Achstetter, Tilman
PA Transgene S.A. Fr
SO Eur Pat Appl, 38 pp
COL EN EP KXDW
DT Patent
LA French
FAN CNT 3

PATENT NO	KIND DATE	APPLICATION NO	DATE
PI EP 607080	A1 19940720	EP 1994-400062	19940111

EP 607080 B1 20010620

R AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

FR 2700338 A1 19940713 FR 1993-171 19930111

FR 2700338 B1 19950331

AT 272381 E 20010715 AT 1994-400062 19940111

ES 2757963 T3 20010901 ES 1994-400062 19940111

WO 9501431 A1 19950112 WO 1994-FR780 19940628

W AL CA JP JS

RW AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

AU 9471282 A1 19950124 AU 1994-71282 19940628

AU 696454 B2 19980910

EP 706567 A1 19960417 EP 1994-920523 19940628

EP 706567 B1 20010609

R AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

JP 09500265 T2 19970114 JP 1994-503310 19940628

ES 2757261 T3 20010816 ES 1994-920523 19940628

US 6077827 A 20000620 US 1985-578674 19951228

US 6277822 B1 20010816 US 2000-498346 20000204

US 2702111259 A1 20020815 US 2001-909950 20010723

PRAI FR 1993-71 A 19930111

FR 1993-790 A 19930629

EP 1994-400062 A 19940111

FR 1994-202 A 19940111

WO 1994-FR780 W 19940628

US 1995-578674 A3 19951228

US 2000-498346 A3 20000204

OS MARPAT 122 2781

AB A sequence encoding the precursor peptide of defensin A of *Phormia terrae novae* is used in expression cassettes to direct secretion of heterologous proteins from yeasts, esp. *Saccharomyces cerevisiae*. A construct that encoded (N to C) the sequence Ser-Leu-Asp-Lys-Arg (C terminus of yeast *MF* *alpha* *gene*), the defensin A pro sequence, and the Lys-47 analog of hirudin HV2 was prepd and placed under control of the *MF* *alpha* *gene* promoter. *S. cerevisiae* transformed with this gene gave a titer of HV2 of 25.6 anti-thrombotic units/A600nm using an expression vector carrying a functional *KEX2* gene, and 8.6 anti-thrombotic units/A600nm without the *KEX2* gene

L4 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2003 ACS

AN 1993 96649 CAPLUS

DN 118 66649

TI Secretory manufacture of human serum albumin with methylotrophic yeasts

IN Davis Geneva Ruth, Provow, Sally Ann

PA Sak Institute Biotechnology/Industrial Assoc., Inc. USA

SO PCT Int Appl, 74 pp

CODEN PI*02

DT Patent

LA English

FAN CNT 1

PATENT NO	KIND DATE	APPLICATION NO	DATE
PI WO 9213951	A1 19920820	WO 1992-US1015	19920204

W JP

PRAI US 1991-650C40 19910204

AB Human serum albumin (HSA) is manu'd in a *methylophilic* *yeast* *Pichia pastoris* by expression of the gene from a MeOH-responsive promoter and the use of *Saccharomyces* or human secretory signals to ensure efficient secretion. The promoter of the *P. pastoris* *AOX1* gene and the signal sequence from the *S. cerevisiae* *alpha*-mating factor gene or the human serum albumin gene signal sequence are used and the expression construct is integrated into the host genome. A synthetic gene for HSA with codon usage optimized for expression in *P. pastoris* was constructed by std. methods and placed under control of the *AOX1* promoter with the human or yeast signal sequence. Different vectors had different copy nos. of the expression cassette. The vector contains a sequence that directs integration into the HIS4 gene of *P. pastoris*. Ferment. regimes that maximized biomass yield by growth on glycerol as C source followed by induction with MeOH efficiently yielded cross-reacting material of the correct mol wt

L4 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC DUPLICATE

3

AN 1992 302938 BIOSIS

DN BA94 16088

TI EXPRESSION AND SECRETION OF HUMAN GROWTH HORMONE IN THE *methylophilic* *yeast* *Hansenula polymorpha*

AU APRIK YAN P G, KARPYCHEV I V, MIKHAILOVER V M, GRACHEVA V D, SHCHEDRIN A M,

BEUROV M YU, EL'DAROV M A, SKRYABIN K G

CS ENG CENT "BIOENG", ACAD SCI RUSS, MOSCOW RUSS

SO DOPLAKAD NAUK SSSR, (1991) 321 (2), 390-394

CODEN DANKAS ISSN 0002-3264

FS BA OLD

LA Russian

AB Regularities in the biosynthesis and secretion of human growth hormone [hGH] were studied in a recombinant *H. polymorpha* strain carrying hGH gene controlled by the promoter and terminator zones of methanol oxidase gene and signal sequence of the ***MF*** **alpha*** **1*** gene of *Saccharomyces cerevisiae* sex pheromone. Data were presented on the ELISA determination of hGH concentrations in media and in cells. It was shown that the presence of a single copy of integrated recombinant plasmid pHP alpha H is sufficient for maintaining a higher level of expression and efficient secretion of hGH.

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NEWS 7 Apr 22 BIOSIS Gene Names now available in TOXCENTER
NEWS 8 Apr 22 Federal Research in Progress (FEDRIP) now available
NEWS 9 Jun 03 New e-mail delivery for search results now available
NEWS 10 Jun 10 MEDLINE Reload
NEWS 11 Jun 10 PCTFULL has been reloaded
NEWS 12 Jul 02 FOREGE no longer contains STANDARDS file segment
NEWS 13 Jul 22 USAN to be reloaded July 28, 2002:
saved answer sets no longer valid
NEWS 14 Jul 29 Enhanced polymer searching in REGISTRY
NEWS 15 Jul 30 NETFIRST to be removed from STN
NEWS 16 Aug 08 CANCERLIT reload
NEWS 17 Aug 08 PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18 Aug 08 NTIS has been reloaded and enhanced
NEWS 19 Aug 19 Aquatic Toxicity Information Retrieval (AQUIRE)
now available on STN
NEWS 20 Aug 19 IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21 Aug 19 The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22 Aug 26 Sequence searching in REGISTRY enhanced
NEWS 23 Sep 03 JAPIO has been reloaded and enhanced
NEWS 24 Sep 16 Experimental properties added to the REGISTRY file
NEWS 25 Sep 16 CA Section Thesaurus available in CAPLUS and CA
NEWS 26 Oct 01 CASREACT Enriched with Reactions from 1907 to 1985
NEWS 27 Oct 21 EVENTLINE has been reloaded
NEWS 28 Oct 24 BEILSTEIN adds new search fields
NEWS 29 Oct 24 Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 30 Oct 25 MEDLINE SDI run of October 8, 2002
NEWS 31 Nov 18 DKILIT has been renamed APOLLIT
NEWS 32 Nov 25 More calculated properties added to REGISTRY
NEWS 33 Dec 02 TBKAT will be removed from STN
NEWS 34 Dec 04 CSA files on STN
NEWS 35 Dec 17 PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 36 Dec 17 TOXCENTER enhanced with additional content
NEWS 37 Dec 17 Adis Clinical Trials Insight now available on STN
NEWS 38 Dec 30 ISMEC no longer available
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AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002

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or alpha1 factor
L1 496 ALPHA PHEROMONE OR MFALPHA1 OR MF ALPHA1 OR MF
A_PHA 1 OR ALPHA
1 FACTOR OR ALPHA1 FACTOR

=> s 1 and (AOX1 (3s) promoter or term?)
L2 162 L1 AND (AOX1 (3S) PROMOTER OR TERM?)

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3 96 DUP REM L2 (66 DUPLICATES REMOVED)

=> s l3 and py<=1999
2 F LES SEARCHED
L4 78 L3 AND PY<=1999

=> s l4 and ptn
L5 0 L4 AND PTN

=> d txb abs l4 1-10

L4 ANSWER 1 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC

AN 1999 527694 BIOSIS
DN PREV199900527694

T: Novel secretion system of recombinant *Saccharomyces cerevisiae* using an N-
terminus residue of human IL-1beta as secretion enhancer.

AU Lee, Jeewon, Choi, Seong-Il, Jang, Jun Sung, Jang, Kyoung, Moon, Jae
Woong, Bae, Cheon Soon, Yang, Doo Suk, Seong, Baik Lin (1)

CS (1) Department of Biotechnology, College of Engineering and Bioproducts
Research Center, Yonsei University, Seoul, 120-749 South Korea

SO Biotechnology Progress (***Sept. Oct., 1999***) Vol. 15, No. 5, pp
884-890

ISSN 8756-7938

DT Article

LA English

SL English

AB An N- **terminus** sequence of human interleukin 1beta (hIL-1beta)

was used as a fusion expression partner for the production of two recombinant therapeutic proteins, human granulocyte-colony stimulating factor (hG-CSF) and human growth hormone (hGH), using *Saccharomyces cerevisiae* as a host. The expression cassette comprised the leader sequence of killer toxin of *Kluyveromyces fragilis*, the N- **terminus** 24 amino acids (Ser5-Ala28) of mature hIL-1beta, the KEX2 dibasic endopeptidase cleavage site, and the target protein (hG-CSF or hGH). The gene expression was controlled by the inducible UASgal/ **MF** - **alpha1** promoter. With the expression vector above, both recombinant proteins were well secreted into culture medium with high secretion efficiencies, and especially, the recombinant hGH was accumulated up to around 1.3 g/L in the culture broth. This is due presumably to the significant role of fused hIL-1beta as secretion enhancer in the yeast secretory pathway. In our recent report, various immunoblotting analyses have shown that the presence of a core N-glycosylation resident in the hIL-1beta fragment is likely to be of crucial importance in the high-level secretion of hG-CSF from the recombinant *S. cerevisiae*. When the N-glycosylation was completely blocked with the addition of tunicamycin to the culture, the secretion of hG-CSF and hGH was decreased to a negligible level although the other host-derived proteins were well secreted to the culture broth regardless

- of the presence of tunicamycin. The N- ***terminal*** sequencing of the purified hG-CSF verified that the hIL-1beta fusion peptide was correctly removed by in vivo KEX2 protease upon the exit of fusion protein from Golgi complex. From the results presented in this article, it is strongly suggested that the N- ***terminus*** fusion of the hIL-1beta peptide could be utilized as a potent secretion enhancer in the expression systems designed for the secretory production of other heterologous proteins from *S. cerevisiae*.
- L4 ANSWER 2 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
AN 1997 454294 BIOSIS
CN PREV199799753497
TI Isolation and characterization of kar2-404 mutation in *Saccharomyces cerevisiae*
AU Kawamura-Watabe, Akiko, Tokunaga, Masao (1)
CS (1) Mitsubishi Kasei Inst. Life Sci., 11 Minamiooya, Machida-shi, Tokyo 194 Japan
SO Bioscience Biotechnology and Biochemistry, (1997) Vol. 61, No. 7, pp. 1172-1178
ISSN 0916-8451
CT Article
LA English
AB We have devised a direct screening method to isolate mutations in the KAR2 gene, and have isolated a B/P/KAR2 mutant, kar2-404, from *Saccharomyces cerevisiae* as a small halo-forming mutant of secreted mouse alpha-amylase. The mutation site was identified as a point mutation at t1337 to c1337 resulting in the Ile404Thr mutation of mature Kar2404p, located at the most NH-2- ***terminal*** first beta-sheet structure (beta-1) of the putative peptide-binding domain. This isoleucine is highly conserved in the Hsp70 family. By pulse-chase experiments, no obvious difference was detected in the intracellular secretion rate of ***MF*** - ***alpha*** - ***1*** -prepro-signal-mouse-alpha-amylase between the wild type and the kar2-404 mutant. However, only about half the amount of secreted heterologous protein, mouse alpha-amylase, was detected in the mutant culture medium compared with wild type. A smaller amount of homologous protein, alpha-factor, was also detected and decreased faster in the mutant culture medium than in wild type. Kar2404p was expressed about 3-fold more than wild type Kar2p, probably to cover its defective functions, and the turnover rates of Kar2p and Kar2-404p were about the same in vivo. The purified Kar2-404p was slightly more sensitive to chymotryptic digestion than Kar2p in vitro.
- L4 ANSWER 3 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
AN 1997 365427 BIOSIS
CN PREV199799657360
TI Over-expression of the *Saccharomyces cerevisiae* exo-beta-1,3-glucanase gene together with the *Bacillus subtilis* endo-beta-1,3-1,4-glucanase gene and the *Butyrivibrio fibrisolvens* endo-beta-1,4-glucanase gene in yeast
AU Van Rensburg, Pierre, Van Zyl, Willem H., Pretorius, Isak S. (1)
CS (1) Inst. Wine Biotechnol., Dep. Microbiol., Univ. Stellenbosch, Stellenbosch 7600 South Africa
SO Journal of Biotechnology, (1997) Vol. 55, No. 1, pp. 43-53
ISSN 0168-1656
CT Article
LA English
AB The EXG1 gene encoding the main *Saccharomyces cerevisiae* exo-beta-1,3-glucanase was cloned and over-expressed in yeast. The *Bacillus subtilis* endo-1,3-1,4-beta-glucanase gene (beg1) and the *Butyrivibrio fibrisolvens* endo-beta-1,4-glucanase gene (end1) were fused to the secretion signal sequence of the yeast mating pheromone alpha-factor (***MF*** - ***alpha*** - ***1*** -S) and inserted between the yeast alcohol dehydrogenase II gene promoter (ADH2p) and ***terminator*** (ADH-2-T). Constructs ADH2-PMF-alpha-1-S-beg1-ADH2-T and ADH2p-***MF*** - ***alpha*** - ***1*** -S-end1-ADH2-T, designated BEG1 and END1, respectively, were expressed separately and jointly with EXG1 in *S. cerevisiae*. The construction of fur1 ura3 *S. cerevisiae* strains allowed for the autoselection of these multicopy URA3-based plasmids in rich medium. Enzyme assays confirmed that co-expression of EXG1, BEG1 and END1 enhanced glucan degradation by *S. cerevisiae*.
- L4 ANSWER 4 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
AN 1997 226684 BIOSIS
CN PREV199799518400
TI Cloning of the *Bacillus pumilus* beta-xylosidase gene (xynB) and its expression in *Saccharomyces cerevisiae*
AU La Grange, D. C., Pretorius, I. S., Van Zyl, W. H. (1)
CS (1) Dep. Microbiol., Univ. Stellenbosch, Victoria St., Stellenbosch 7600 South Africa
SO Applied Microbiology and Biotechnology, (1997) Vol. 47, No. 3, pp. 262-266
ISSN 0175-7598
CT Article
LA English
AB A genomic DNA library of the bacterium *Bacillus pumilus* PLS was constructed and the beta-xylosidase gene (xynB) was amplified from a 3-kb genomic DNA fragment with the aid of the polymerase chain reaction technique. The amplified xynB gene was inserted between the yeast alcohol dehydrogenase II gene promoter (ADH2-P) and ***terminator*** (ADH2-T) sequences on a multicopy episomal plasmid (pDLG11). The xynB gene was also fused in-frame to the secretion signal sequence of the yeast mating pheromone alpha-factor (***MF*** - ***alpha*** - ***1*** -S) before insertion between the ADH2-P and ADH2-T sequences on a similar multicopy episomal plasmid (pDLG12). The resulting construct ADH2-P-***MF*** - ***alpha*** - ***1*** -S-xynB-ADH2-T was designated XLO1. Both plasmids pDLG11 and pDLG12 were introduced into *Saccharomyces cerevisiae* but only the expression of the XLO1 gene yielded biologically functional beta-xylosidase. The total beta-xylosidase activity remained cell-associated with a maximum activity of 0.09 nkat/ml obtained when the recombinant *S. cerevisiae* strain was grown for 143 h in synthetic medium. The temperature and pH optima of the recombinant Xlo1 enzyme were 45-50 degree C and pH 6.6 respectively. The enzyme was thermostable at 45 degree C; however, at 60 degree C most of the Xlo1 was inactive after 5 min.
- L4 ANSWER 5 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
AN 1996 484724 BIOSIS
CN PREV199699199980
TI Co-expression of a *Phanerochaete chrysosporium* cellobiohydrolase gene and a *Butyrivibrio fibrisolvens* endo-beta-1,4-glucanase gene in *Saccharomyces cerevisiae*
AU Van Rensburg, Pierre, Van Zyl, Willem H., Pretorius, Isak S. (1)
CS (1) Dep. Microbiol., Inst. Wine Biotechnol., Univ. Stellenbosch, Stellenbosch 7600 South Africa
SO Current Genetics, (1996) Vol. 30, No. 3, pp. 246-250
ISSN 0172-8083
CT Article
LA English
AB A cDNA fragment encoding the *Phanerochaete chrysosporium* cellobiohydrolase (cbh1-4) was amplified and cloned with the aid of the polymerase chain reaction (PCR) technique. The cbh1-4 gene and the *Butyrivibrio fibrisolvens* endo-beta-1,4-glucanase (end1) gene were successfully expressed in *Saccharomyces cerevisiae* under the control of the phosphoglycerate kinase-I (PGK1) and alcohol dehydrogenase-II (ADH2) gene promoters and ***terminators***, respectively. The native *P. chrysosporium* signal sequence mediated secretion of cellobiohydrolase in *S. cerevisiae*, whereas secretion of the endo-beta-1,4-glucanase was directed by the signal sequence of the yeast mating pheromone alpha-factor (***MF*** - ***alpha*** - ***1*** -S). These constructs, designated CBH1 and END1, respectively, were expressed separately and jointly in *S. cerevisiae*. The construction of fur1 ura3 *S. cerevisiae* strains allowed for the autoselection of these multicopy URA3-based plasmids in rich medium. Enzyme assays confirmed that co-expression of CBH1 and END1 synergistically enhanced cellulose degradation by *S. cerevisiae*.
- L4 ANSWER 6 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
AN 1996 121990 BIOSIS
CN PREV199698694125
TI Recombinant outer-surface protein A (des-Cys-1-OspA) from the Lyme disease spirochete *Borrelia burgdorferi*: High production levels in *Saccharomyces cerevisiae* yeast cultures
AU Mendoza-Vega, O. (1), Keppl, E., Bouchon, B., Nguyen, M., Achstetter, T.
CS (1) Transgene S.A., 11 rue Molshiem 67082 Strasbourg Cedex France
SO Applied Microbiology and Biotechnology, (1996) Vol. 44, No. 5, pp. 624-628
ISSN 0175-7598
CT Article
LA English
AB The recombinant outer-surface protein A with an N- ***terminally*** truncated form (des-Cys-1-OspA) from the Lyme disease spirochete *Borrelia burgdorferi* was expressed in *Saccharomyces cerevisiae* at high production levels. Since the recombinant vaccine candidate expressed in *Escherichia coli* exhibits low production yields and the purification of lipoproteins appears to be difficult, we have investigated the secretion of a soluble recombinant OspA in the yeast *S. cerevisiae*. In this way, a Leu+ derivative of *S. cerevisiae* c13ABYS86 was used as the host strain transformed with an expression plasmid containing the gene encoding des-Cys-1-OspA and driven by the ***MF*** - ***alpha*** - ***1*** promoter. The fed-batch culture results revealed that an efficient secretion of des-Cys-1-OspA is obtained with a high production level of about 2.1 g/l at a cell density of 101 g/l cell dry weight. The accumulation of recombinant protein in the supernatant exceeds 6% of the total yeast proteins when estimated by sodium dodecyl sulphate/polyacrylamide gel electrophoresis. Moreover, des-Cys-1-OspA showed lower solubilities at high cell densities and, as a consequence, a fraction of the recombinant protein precipitated. An internal cleavage of the ***MF*** - ***alpha*** - ***1*** pro-des-Cys-1-OspA precursor was also detected. However, in this case the cleavage occurred at a frequency such that the large amounts of the secreted des-Cys-1-OspA could be employed for the evaluation of an immunogenic effect on animal immunization. These studies will extend the knowledge of the usefulness of OspA as a vaccine for Lyme borreliosis.
- L4 ANSWER 7 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
AN 1996 106477 BIOSIS
CN PREV199698678612
TI Highly efficient secretion of heterologous proteins from *Saccharomyces cerevisiae* using inulinase signal peptides

- AU: Chung, Bong Hyun (1); Nam, Soo Wan; Kim, Byung Moon; Park, Young Hoon
 CS: (1) Korea Res. Inst. Biosci., Biotechnol. P.O. Box 115, Yusong, Taejeon 305-600 South Korea
 SO: Biotechnology and Bioengineering, (1996) Vol. 49, No. 4, pp. 473-479
 ISSN: 0006-3592
 DT: Article
 LA: English
 AB: The INU genes of *Kluyveromyces marxianus* encode inulinases which are readily secreted from *Saccharomyces cerevisiae* into the culture medium. To evaluate the utility of the INU signal peptides for the secretion of heterologous proteins from *S. cerevisiae*, a variety of expression and secretion vectors were constructed with GA₁₀ promoter and GAL7 ***terminator***. The coding sequence for human lipocortin-1 (LC1) was inserted in-frame with the INU signal sequences, and then the secretion efficiency and localization of LC1 were investigated in more detail and compared with those when being expressed by the vector with the ***MF*** - ***alpha*** - ***1*** leader peptide. The vector systems with INU signal peptides secreted ca. 95% of the total LC1 expressed into the extracellular medium, while the ***MF*** - ***alpha*** - ***1*** leader peptide-containing vector resulted in very low secretion efficiency below 10%. In addition, recombinant human interleukin-2 (IL-2) was expressed and secreted with the vector systems with INU signal peptide and a majority fraction of the human IL-2 expressed was found to be secreted into the extracellular medium as observed in LC1 expression.
- L4: ANSWER 8 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
 AN: 1995 361980 BIOSIS
 DN: PREV199598376280
 TI: CycM3, a novel B-type alfa cyclin gene, is induced in the G-0-to-G-1 transition of the cell cycle
 AU: Mesikene, Irute; Bogre, Laszlo; Dahl, Marlis; Pirck, Manfred; Dang Thi Cam Ha; Swoboda, Ines; Heberle-Bors, Erwin; Ammerer, Gustav; Hirt, Heribert (1)
 CS: (1) Vienna Biocent., Inst. Microbiol. and Genet., Dr. Bohrgasse 9, A-1030 Vienna Austria
 SO: Plant Cell, (1995) Vol. 7, No. 6, pp. 759-771
 ISSN: 1040-4851
 DT: Article
 LA: English
 AB: Cyclins are key regulators of the cell cycle in all eukaryotes. We have previously isolated two B-type cyclin genes, cycM3 and cycM2, from alfa that are primarily expressed during the G-2-to-M phase transition and are most likely mitotic cyclin genes. Here, we report the isolation of a novel alfa cyclin gene, ***termed*** cycM3 (for cyclin Medicago sativa), by selecting for mating type ***alpha*** - ***pheromone*** -induced cell cycle arrest suppression in yeast. The central region of the predicted amino acid sequence of the cycM3 gene is most similar to the cyclin box of yeast B-type and mammalian A- and B-type cyclins. In situ hybridization showed that cycM3 mRNA can be detected only in proliferating cells and not in differentiated alfa cells. When differentiated G-0-arrested cells were induced to reenter the cell cycle in the G-1 phase and resume cell division by treatment with plant hormones, cycM3 transcript levels increased long before the onset of DNA synthesis. In contrast, histone H3-1 mRNA and cycM2 transcripts were not observed before DNA replication and mitosis, respectively. In addition, cycM3 mRNA was found in all stages of the cell cycle in synchronously dividing cells, whereas the cycM2 and histone H3-1 genes showed a G-2-to-M phase- or S phase-specific transcription pattern, respectively. These data suggest that the role of cyclin CycM3 differs from that of CycM1 and CycM2. We propose that CycM3 helps control reentry of quiescent G-0-arrested cells into the G-1 phase of the cell cycle.
- L4: ANSWER 9 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
 AN: 1995 205176 BIOSIS
 DN: PREV199598219476
 TI: One-step enzymatic hydrolysis of starch using a recombinant strain of *Saccharomyces cerevisiae* producing alpha-amylase, glucoamylase and pullulanase
 AU: Janse, B. J. H.; Pretorius, I. S. (1)
 CS: (1) Inst. Biotechnol., Univ. Stellenbosch, Stellenbosch South Africa
 SO: Applied Microbiology and Biotechnology, (1995) Vol. 42, No. 6, pp. 878-883
 ISSN: 0175-7598
 DT: Article
 LA: English
 AB: A recombinant strain of *Saccharomyces cerevisiae* was constructed that contained the genes encoding a bacterial alpha-amylase (AMY1), a yeast glucoamylase (STA2) and a bacterial pullulanase (pula). The *Bacillus amyloliquefaciens* alpha-amylase and *S. cerevisiae* var. diastaticus glucoamylase genes were expressed in *S. cerevisiae* using their native promoters and the encoded enzymes secreted under direction of their native leader sequences. In contrast, the *Klebsiella pneumoniae* pullulanase gene was placed under the control of the yeast alcohol dehydrogenase gene promoter (ADC1-P) and secreted using the yeast mating pheromone alpha-factor secretion signal (***MF*** - ***alpha*** - ***1*** -S). Transcription ***terminator*** of the pullulanase gene was effected by the yeast tryptophan synthase gene ***terminator*** (TRP5-T) whereas ***terminator*** of the glucoamylase and alpha-amylase genes was directed by their native ***terminators***. Pullulanase (PUL1) produced by recombinant yeasts containing ADC1-P ***MF*** - ***alpha*** - ***1*** -S pula TRP5-T (designated PUL1) was further characterized and compared to its bacterial counterpart (Pu1A). The different genes were introduced into *S. cerevisiae* in different combinations and the various amyolytic *Saccharomyces* transformants compared to *Schwanniomyces occidentalis*. Introduction of PUL1 into a *S. cerevisiae* strain containing both STA2 and AMY1 resulted in 99% assimilation of starch.
- L4: ANSWER 10 OF 78 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC
 AN: 1995 108081 BIOSIS
 DN: PREV199598122381
 TI: *Saccharomyces cerevisiae* CNE1 Encodes an Endoplasmic Reticulum (ER) Membrane Protein with Sequence Similarity to Calnexin and Calreticulin and Functions as a Constituent of the ER Quality Control Apparatus
 AU: Parlati, Francesco; Dominguez, Michel; Bergeron, John J. M.; Thomas, David Y. (1)
 CS: (1) Natl. Res. Council Canada, Biotechnol. Res. Inst., 6100 Royalmount Ave., Montreal, PQ H4P 2R2 Canada
 SO: Journal of Biological Chemistry, (1995) Vol. 270, No. 1, pp. 244-253
 ISSN: 0021-9258
 DT: Article
 LA: English
 AB: We have used a polymerase chain reaction strategy to identify in the yeast *Saccharomyces cerevisiae* genes of the mammalian calnexin/calreticulin family, and we have identified and isolated a single gene, CNE1. The protein predicted from the CNE1 DNA sequence shares some of the motifs with calnexin and calreticulin, and it is 24% identical and 31% similar at the amino acid level with mammalian calnexin. On the basis of its solubility in detergents and its lack of extraction from membranes by 2.5 M urea, high salt, and sodium carbonate at pH 11.5, we have established that Cne1p is an integral membrane protein. However, unlike calnexins, the predicted carboxyl- ***terminal*** membrane-spanning domain of Cne1p ***terminates*** directly. Furthermore, based on its changed mobility from 76 to 60 kDa after endoglycosidase H digestion Cne1p was shown to be N-glycosylated. Localization of the Cne1p protein by differential and analytical subcellular fractionation as well as by confocal immunofluorescence microscopy showed that it was exclusively located in the endoplasmic reticulum (ER), despite the lack of known ER retention motifs. Although six Ca²⁺-binding proteins were detected in the ER fractions, they were all soluble proteins, and Ca²⁺ binding activity has not been detected for Cne1p. Disruption of the CNE1 gene did not lead to inviable cells or to gross effects on the levels of secreted proteins such as ***alpha*** - ***pheromone*** or acid phosphatase. However, in CNE1 disrupted cells, there was an increase of cell-surface expression of an ER retained temperature-sensitive mutant of the ***alpha*** - ***pheromone*** receptor, ste2-3p, and also an increase in the secretion of heterologously expressed mammalian alpha-1 antitrypsin. Hence, Cne1p appears to function as a constituent of the *S. cerevisiae* ER protein quality control apparatus.
- => s11 and (AOX1 (3a) promoter or term?)
 L6 162 L1 AND (AOX1 (3A) PROMOTER OR "ERM")
 => dup rem l6
 PROCESSING COMPLETED FOR L6
 L7 96 DUP REM L6 (66 DUPLICATES REMOVED)
 => s AOX1 (3a) (promoter or termina?)
 L8 300 AOX1 (3A) (PROMOTER OR TERMINA?)
 => s11 and l8
 L9 4 L1 AND L8
 => dup rem l9
 PROCESSING COMPLETED FOR L9
 L10 4 DUP REM L9 (0 DUPLICATES REMOVED)
 => d bib abs 1.
 YOU HAVE REQUESTED DATA FROM 4 ANSWERS. - CONTINUE? Y/(N) y
- L10: ANSWER 1 OF 4 CAPLUS COPYRIGHT 2003 ACS
 AN: 2002 57522 CAPLUS
 DN: 137 136056
 TI: Combinatorial expression libraries with individual members of the library containing concatamers of expression cassettes
 IN: Goldsmith, Neil; Sorensen, Alexandra M. P.; Santana, Nelsen, Soren V. S.
 PA: Evolve Biotech A/S, Den.
 SO: PCT Int. App., 115 pp
 CODEN: PIXX02
 DT: Patent
 LA: English
 FAN CNT 1
 PATENT NO. KIND DATE APPLICATION NO. DATE
 WO 2002059297 A2 20020801 WO 2002.056 20020125
 W AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, F, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NC, NZ, OM, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI/DK 2001-128 A 20010125
DK 2001-679 A 20010501
US 2001-300863P P 20010627

AB Combinatorial gene expression libraries in which recombination between individual sequences can take place within an individual cell and methods of constructing such libraries are described. Each member of the library contains a large no. of expression cassettes that are randomly selected from a pool of cassettes during the construction of the library. Individual expression cassettes are flanked by a common pair of restriction sites and have the same promoter and terminator to regulate expression of the cloned inserts. The library of concatemers is created from a library of individual clones. This primary library, typically a cDNA library, has the individual cassette and its flanking restriction sites flanked by a second pair of restriction sites. The cassettes are released from the library and ligated into concatemers that are then cloned into a vector capable of stabilizing large inserts, esp. artificial chromosomes. The variability within the combinatorial library can be increased by using cDNA libraries from multiple sources. Cassettes within the library are free to recombine with one another to create genes encoding novel activities or functions that can be identified by selection or screening. Such libraries are useful in discovery of novel or modified metabolic pathways leading to the production of novel compounds, for e.g. drug discovery and to the production of known compounds in novel quantities or in novel compartments of the cells. The expression libraries in particular are composed of host cells capable of coordinated and controllable expression of large nos. of heterologous genes in the host cells.

L10 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS

AN 2002 575221 CAPLUS

DN 137 136055

TI Combinatorial expression libraries with individual members of the library containing concatemers of expression cassettes

IN Goldsmith, Neil; Sorensen, Alexandria M. P.; Santana, Nielsen, Soren V. S.; Naesby, Michael

PA Evolve Biotech A/S, Den

SO PCT Int. Appl., 124 pp

CODEN PIXXD2

DT Patent

LA English

FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
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PI WO 2002059296 A2 20020801 WO 2002-0155 20020125
W AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, JZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI/DK 2001-127 A 20010125
US 2001-301022P P 20010627

AB Combinatorial gene expression libraries in which individual clones contain large nos. of expression cassettes and methods of constructing such libraries are described. Each member of the library contains a large no. of expression cassettes that are randomly selected from a pool of cassettes during the construction of the library. Individual expression cassettes are flanked by a common pair of restriction sites and have the same promoter and terminator for uniform regulation of expression of the cloned inserts. The library of concatemers is created from a library of individual clones. This primary library, typically a cDNA library, has the individual cassette and its flanking restriction sites flanked by a second pair of restriction sites. The cassettes are released from the library and ligated into concatemers that are then cloned into a vector capable of stabilizing large inserts, esp. artificial chromosomes. The variability within the combinatorial library can be increased by using cDNA libraries from multiple sources. Such libraries are useful in discovery of novel or modified metabolic pathways leading to the production of novel compounds, for e.g. drug discovery and to the production of known compounds in novel quantities or in novel compartments of the cells. The expression libraries in particular are composed of host cells capable of coordinated and controllable expression of large nos. of heterologous genes in the host cells.

L10 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS

AN 2002 133856 CAPLUS

DN 137 179658

TI Mass secretion/expression system for unglycosylated human MK family proteins in methylotrophic yeast

IN Murasugi, Akira; Asami, Yukio; Kido, Isao; Kumai, Hideshi

PA Meiji Milk Products Co., Ltd., Japan

SO PCT Int. Appl., 58 pp

CODEN PIXXD2

DT Patent

LA Japanese

FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
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PI WO 2000003718 A1 20000224 WO 1999-JP4332 19990810
W AU, CA, CN, JP, KR, US
RW AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

CA 2339350 AA 20000224 CA 1999-2339350 19990810
AU 9950674 A1 20000306 AU 1999-50674 19990810
EP 1106697 A1 20010613 EP 1999-935123 19990810

R AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRAI JP 1998-236621 A 19980810

JP 1999-84533 A 19990326

WO 1999-JP4332 W 19990810

AB Large-scale secretion/expression system for unglycosylated human MK family proteins in methylotrophic yeast, *Pichia pastoris*, is described. The expression vector consists of a mature human MK family protein coding region cDNA attached to *Saccharomyces cerevisiae* ***alpha*** ***1*** ***factor*** signal sequence under the control of a *Pichia pastoris* methanol-inducible alc oxidase gene (***AOX1***). ***promoter***, a transcription ***termination*** sequence, and an origin of replication. Unglycosylated human MK protein and PTN protein were mass produced in *Pichia pastoris* GS115 and SMD118 strains, and the expressed proteins demonstrated their biological activity for facilitating growth of mouse embryo fibroblast NIH3T3.

RE CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE REFORMAT

L10 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS

AN 1993 96649 CAPLUS

DN 118 96649

TI Secretory manufacture of human serum albumin with methylotrophic yeasts

IN Davis, Geneva Ruth; Provost, Sally Ann

PA Salk Institute Biotechnology/Industrial Assoc., Inc., USA

SO PCT Int. Appl. 74 pp

CODEN PIXXD2

DT Patent

LA English

FAN CNT 1

PATENT NO	KIND	DATE	APPLICATION NO	DATE
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PI WO 9213951 A1 19920820 WO 1992-US1015 19920204
W JP

PRA US 1991-650J40 19910204

AB Human serum albumin (HSA) is manufactured in a methylotrophic yeast (*Pichia pastoris*) by expression of the gene from a MeOH-responsive promoter and the use of *Saccharomyces* or human secretory signals to ensure efficient secretion. The promoter of the *P. pastoris* alc oxidase gene (AOX1) gene and the signal sequence from the *S. cerevisiae* alpha-mating factor gene or the human serum albumin gene signal sequence are used and the expression construct is integrated into the host genome. A synthetic gene for HSA with codon usage optimized for expression in *Pichia* was constructed by standard methods and placed under control of the ***AOX1*** ***promoter*** with the human or yeast signal sequence; different vectors had different copy nos. of the expression cassette. The vector contains a sequence that directs integration into the HIS4 gene of *Pichia*. Fermentation regimes that maximized biomass yield by growth on glycerol as C source followed by induction with MeOH efficiently yielded cross-reacting material of the correct molecular weight.

=> d his

(FILE HOME ENTERED AT 13 43 45 ON 29 JAN 2003)

FILE BIOSIS EMBASE CAPLUS ENTERED AT 13 43 58 ON 29 JAN 2003

L1 496 S ALPHA-PHEROMONE OR MFALPHA1 OR MF ALPHA1 OR MF ALPHA1 OR ALP

L2 162 S L1 AND (AOX1 (3S) PROMOTER OR TERM?)

L3 96 DUP REM L2 (66 DUPLICATES REMOVED)

L4 78 S L3 AND PY<-1999

L5 0 S L4 AND PTN

L6 162 S L1 AND (AOX1 (3A) PROMOTER OR TERM?)

L7 96 DUP REM L6 (66 DUPLICATES REMOVED)

L8 300 S AOX1 (3A) (PROMOTER OR TERMINA?)

L9 4 S L1 AND L8

L10 4 DUP REM L9 (0 DUPLICATES REMOVED)

=> s l1 and (AOX1 (3s) (promoter or term?))

L11 4 L1 AND (AOX1 (3S) (PROMOTER OR TERM?))

=> s l11 not l10

L12 0 L11 NOT L10

=> d his

(FILE HOME ENTERED AT 13 43 45 ON 29 JAN 2003)

FILE BIOSIS EMBASE CAPLUS ENTERED AT 13 43 58 ON 29 JAN 2003

L1 496 S ALPHA-PHEROMONE OR MFALPHA1 OR MF ALPHA1 OR MF ALPHA1 OR ALP

L2 162 S L1 AND (AOX1 (3S) PROMOTER OR TERM?)

L3 96 DUP REM L2 (66 DUPLICATES REMOVED)

L4 78 S L3 AND PY<=1999
L5 0 S L4 AND PTN
L6 162 S L1 AND (AOX1 (3A) PROMOTER OR TERM?)
L7 96 DUP REM L6 (66 DUPLICATES REMOVED)
L8 300 S AOX1 (3A) (PROMOTER OR TERMINA?)
L9 4 S L1 AND L8
L10 4 DUP REM L9 (4 DUPLICATES REMOVED)
L11 4 S L1 AND (AOX1 (3S) (PROMOTER OR TERM?))
L12 0 S L11 NOT L10

=>

---Logging off of STN---

=>

Executing the logoff script

=> LOG Y

COST IN U S DOLLARS	ENTRY	SINCE FILE SESSION	TOTAL
FULL ESTIMATED COST		88 66	88 87
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE			
TOTAL	ENTRY	SESSION	
CA SUBSCRIBER PRICE		-2 60	-2 60

STN INTERNATIONAL LOGOFF AT 13 53 14 ON 29 JAN 2003